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Antioxidant activity derived from *Punica granatum L.* peels extract in micetoxicity induced by a mixture of *Nerium oleander* extract, acetaminophen and gentamicin

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ABSTRACT

Reactive oxygen species (ROS) are formed through the body's metabolism. Uncontrolled production of ROS occurs during oxidative stress-induced in various diseases, such as diabetes mellitus. Polyphenolic compounds are widely recognized to possess a wide range of biological properties including anti-inflammatory and anti-thrombotic properties including. This study was aimed to evaluate the antioxidant activity of the *Punica granatum L.* peels extract in mice toxicity induced by a mixture of Nerium oleander extracts, acetaminophen and gentamicin. *Methods:* 40 mice were classified into four groups: Group 1 (control group), Group 2 (*Punica granatum L.* cortex control group), Group 3 (toxin control group), Group 4 (toxin and *Punica granatum L.* cortex extract). Weights of the mice showed significant differences between all groups ($p < 0.05$). The serum HDL-C level was higher in Group 2 than control group ($p < 0.05$). Biochemical markers were significantly decreased in Group 4 compared with toxins group ($p<0.01$). In cardiac tissue, GSH-Px, GSH, and catalase levels increased in Group 4 compared with its level in the toxins group ($p < 0.01$, $p < 0.05$, and $p<0.05$). In hepatic and kidney tissues, catalase and GSH-Px levels had been higher in Group 4 compared with toxins group ($p < 0.01$ and $p < 0.05$). *Conclusion: Punica granatum L.* peels induced hypolipidemic effect. This effect might be anticipated to the potential antioxidant effect against cholesterol oxidation. There is a protective effect of the extract on liver histology against mixture of toxins. Also, the extract protects the heart from necrosis and massive haemorrhage.

Keywords *Punica granatum L.*, *Nerium oleander*, oxidation stress, antioxidants.

1. INTRODUCTION

Daily, ROS are formed through the body's metabolism. These molecules react rapidly, causing the destruction of macromolecules, such as lipids, proteins, and nucleic acids (Puan and Ismail, 2010). Uncontrolled production of ROS occurs during oxidative stress induced by various diseases, such as diabetes mellitus, inflammatory diseases, and hypertension (Huang et al., 2005). ROS induce cell membrane lipid peroxidation (LPO), leading to cell damage, gene mutation, and tissue injuries (Negi et al., 2003). Antioxidants are the first line of defence against endogenous or exogeneous oxidative stress that might occur. Endogenous antioxidants, for instance, antioxidant enzymes, such as catalase and superoxide dismutase (SOD), are produced by the body. On the other hand, exogenous antioxidants, such as ascorbic acid and flavonoids, can be obtained through dietary intake (Hahr and Molitch, 2015). Most exogenous antioxidants are present in plant-based food (Han et al., 2008). Consumption of these antioxidants counteracts the injuries that generated via oxidative stress or protects the body from its harmful effects. In the past, synthetic antioxidants were used as food supplements to overcome the destructive effect of oxidative stress (Iqbal et al., 2008); however, the use of crude extracts from different medicinal plants was found to be more effective than synthetic antioxidants (Al-Zoreky, 2009). Polyphenolic compounds are one of the many compounds that play a critical role in the body's protection against ROS. These compounds have been widely recognised to possess a wide range of biological properties, including anti-inflammatory, anti-microbial, and anti-thrombotic properties (Reddy et al. 2007).

Pomegranate (*Punica granatum L.*) is a small tree in the *Punicaceae* family; it grows in Asia, Europe, and America (Holland et al., 2009). *Punica granatum L.* is rich in phenolic compounds and other bioactive phytochemicals, such as tannins (punicalagin), ellagic acid, and anthocyanins (Li et al., 2006). It also has high nutritional value because it contains carbohydrates, minerals, and fibres (Zaouay et al., 2012). For several centuries in many civilisations, pomegranate was used in the treatment of gastritis, bronchitis, diarrhoea, dysentery, and urinary tract infections (Reddy et al., 2007). A previous study showed that *Punica granatum L.* extract administration reduced levels of oxidative reaction in coronary arteries (Al-Jarallah et al., 2013). Several studies were conducted to evaluate the antioxidant activity of *Punica granatum L.* juice or extracts with different experimental designs, but the results were contradictory (Chidambara Murthy *et al.*, 2002; Al-Zoreky, 2009; Madrigal-Carballo *et al.*, 2009). This study was aimed to estimate the effect of the antioxidant activity of *Punica granatum L.* peel extract on toxicity in mice induced by a mixture of acetaminophen and gentamicin.

2. MATERIALS AND METHODS

Chemicals

A radioimmunoprecipitation assay (RIPA) buffer and a protease inhibitor were purchased from Thermo Scientific, while a 2 mL bead kit was obtained from Omni international. All colourimetric kits for the estimation of antioxidant enzymes and oxidative stress were purchased from Elabscience Biotechnology Inc.

Collection of pomegranates (*Punica granatum L.*)

Punica granatum L. was collected fresh from local farms of Khiasah District, Baljurashi, Al-Baha province, Saudi Arabia. Fresh fruits were processed at the Medical Research Centre of Umm Al-Qura University, Saudi Arabia.

Preparation of the pomegranate peel extract

Initially, *Punica granatum L.* was washed thoroughly using distilled water. Then, the fruit was peeled, and the white core sacs were removed. Afterwards, the fruit peels were dried in an SVAC4 vacuum oven (Sheldon®, Inc.) at 45°C under 60 mmHg of pressure for 10 hours. The sample was monitored to avoid any contamination. Dried peels were left to cool down at room temperature for 30 minutes before grounding to fine powder using a razor blade. At that point, the sample was stored in a sterile container for further analysis.

Methanolic extraction of *Punica granatum L.* peels

The fine powder (6 g) was submerged in 200 mL freshly prepared 80% methanol (Sajjad et al., 2015). The mixture was incubated for 10 days at room temperature under magnetic steering at 2,000 rpm. The solvent was changed every 24 h and stored in sterile glass (DURAN®, Germany) for further analysis. Afterwards, the sample was sterile-filtered using Whatman® filter paper, and it was evaporated using Eppendorf® Concentrator Plus (Germany) overnight at 45°C.

Preparation of the *Nerium oleander* ethanolic extract

The *Nerium oleander* tree was purchased from a seedling shop. Both pink flowers and green leaves were washed with clean water and then dried at 40°C in a drying oven. The dried flowers and leaves were ground into a fine powder, and 500 g of it was soaked in 90% ethanol and filtered using filter paper. The extract was eventually kept at 5°C in a refrigerator.

Animals

Following the agreement of the Biomedical Research Ethics Committee, Faculty of Medicine, Umm Al-Qura University, the experiment was carried out in accordance with the EU Directive 2010/63/EU recommendations for animal research (ethical approval number: HAPO-02-K-012-2021-03-614). The white BALB/c mice were purchased from a local rodent market with an average weight of 19–21 g and an age of 6–8 weeks. The mice were kept in polycarbonate cages with a wood chip bedding (10 mice per cage). They were kept at a temperature of 32°C in a huge ventilated chamber with an automatic 12-hour light/dark cycle. During the acclimatization period, mice had unlimited access to water and were fed rodent chow.

Experimental design

After two weeks of acclimatization, 40 mice were divided into four groups at random (10 mice each) as follows:

Group 1 (control group): 10 mice received normal mouse food and bottled water.

Group 2 (*Punica granatum L.* cortex control group): 10 mice were fed standard rodent food and were given bottled water. Furthermore, *Punica granatum L.* cortex extract 500 mg/kg body weight was given to the mice every day via intragastric gavage.

Group 3 (toxin control group): 10 mice were fed standard rodent food and ordinary water. Moreover, each mouse was administered *Nerium oleander* ethanolic extract via intragastric gavage two times/week at a dose 200 mg/kg. Finally, 300 mg/kg acetaminophen (Panadol infant drops 100 mg/mL) was given intraperitoneally into the mice, and 50 mg/kg body weight gentamicin (baby drops) two times/weekafter overnight fasting (Kane et al., 2016).

Group 4 (toxin and *Punica granatum L.* cortex extract): 10 mice were fed standard rodent food and ordinary water. Mice were administered *Punica granatum L.* cortex extract in the positive control group 2. Furthermore, the mice were given *Nerium oleander* ethanolic via intragastric gavage two times/week at a 200 mg/kg dosage the mice were given a combination of acetaminophen and gentamicin at the conclusion of the experiment, similarto group 3.

During the experiment period, mice behaviour was observed daily for the presence of abnormal clinical signs.

Collection of blood samples

In the 60th day and after 12 hours of toxin injection, blood samples were obtained from each mouse in plain and EDTA tubes. The serum was immediately used for the estimation of glucose, total cholesterol, high density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglyceride (TGL), urea, and creatinine levels. Moreover, serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total creatinine kinase (CK), CK-MB, and troponin-I levels were measured using the Cobas Integra 400 plus (Roche, Basel, Switzerland). The EDTA samples were used to estimate complete blood count (CBC) using CBC Mindray BC-2800 (Shenzhen, China). Moreover, all mice were sacrificed; then, the kidneys, the liver, and the heart were rapidly excised for biological analyses.

Preparation of tissue homogenates

Tissue homogenates were prepared, including the liver, kidney, and heart using a 2 mL kit-bead (Omni International); 700 µL of a RIPA buffer with 7 µL of a protease inhibitor was added to small pieces of the organ (1 mg) in a 2 mL microtube. The microtube was placed in a homogeniser machine (Read Roptor 12, Omni International) three times for 99 seconds each. Then, a microtube was centrifuged for 30 minutes at -1°C at 15,000 rpm (SIGMA 1-14 k). The supernatant was then transfered into an Eppendorf tube and then centrifuged for 30 minutes at -1°C at 15,000 rpm. The supernatant was extracted and kept at -20°C until further examination.

Estimation of tissue homogenate oxidants and antioxidant parameters

Estimation of lipid peroxidation products

In an acidic media, at 95°C for 30 minutes, thiobarbituric acid reacts with malondialdehyde to estimate LPO, forming a reactive product. The absorbance of a pink-coloured product was measured through a colourimetric assay at 534 nm using Varioskan™ LUX (Thermo Fisher Scientific, USA).

Measurement of carbonyl carbon

Dinitrophenylhydrazine was used to react with the carbonyl carbon (CC) in the sample, forming a Schiff base. This Schiff base produced the corresponding hydrazone measured at 366 nm.

Estimation of reduced glutathione

Glutathione (GSH) levels were estimated via reaction with dinitrobenzoic acid to form a complex to be detected colourimetrically at 405 nm.

Estimation of catalase

Hydrogen peroxide was decomposed by the catalase in the sample, forming a yellowish complex, which can be measured by a colourimetric assay at 405 nm.

Estimation of glutathione peroxidase

Reduced glutathione peroxidase (GSH-Px) residues were obtained via its reaction with dinitrobenzoic acid, producing 5-thio-dinitro benzoic acid anion. This compound showed a yellowish colour measured by a colourimetric assay at 412 nm.

Estimation of superoxide dismutase

The activity of SOD was measured by using the water-soluble tetrazolium 1 (WST-1) method. Oxygen was reacted with WST-1 to generate water-soluble formazan resulted in catalysed xanthine oxidase. The presence of SOD is tended to stop the reaction; therefore, using a spectrophotometer, the reaction level was found to be inversely related to the amount of formazan dye. SOD's response inhibition ratio was estimated as follows: following; i= ([A] control - [A] blank) – ([A] sample - [A] blank) ÷ ([A] control - [A] blank) x 100, where (i) stands for inhibition and (A) stands for absorbance. SOD activity was determined as following T-SOD activity (μg/g protein) = I ÷ 50%×(V1÷V2) × f ÷ protein concentration, where the reaction total volume (V1), the sample volume (V2) that added to the reaction, and f is the dilution factor.

3. RESULTS

At the beginning of the experiment, one month later, and at the completion of the experiment, the weights of all mice were measured (Table 1); at the end of the experiment (after eight weeks), the weights of the mice showed significant differences between all groups ($p<0.05$). The *Punica granatum L.* group had the highest weight compared with the others. The RBC count, haemoglobin concentration, hematocrit, and MCV show no statistically significant differences between the groups (Table 2). Table 3 summarises the serum levels of glucose, triglyceride, cholesterol, HDL-C, LDL-C, creatinine, urea, ALT, AST, ALP, CK, CK-MB, and troponin I. The levels of serum cholesterol and LDL-C were statistically reduced in the *Punica granatum L.* extract group in comparison to a control group ($p<0.05$). On the other hand, significantly high level of serum HDL-C was determined in the *Punica granatum L.* group in comparison to the control group ($p<0.05$). Moreover, the serum urea level was significantly decreased in the *Punica granatum L.* group after toxins injections compared with the toxins group ($p<0.05$). Furthermore, serum CK, CK-MB,ALT and AST activities were significantly decreased in the *Punica granatum L.* group following the administration of toxins compared with the toxins group ($p<0.01$) also, a significantly low level of the serum troponin-I in the *Punica granatum L.* group after toxins injections than that in the toxins group ($p<0.01$) (Table 3).

Table 1 Mice body weight across the groups and the across the time scale

	Control group	*Punica granatum L.* control group	Toxin group	*Punica granatum L.* + toxin group	*p* value
At the beginning of experiment	19.96±1.37	20.19±1.09	19.87±1.22	20.08±1.37	0.102
After four weeks	21.82±1.69	23.64±1.71	19.06±1.03	20.60±1.93	0.086
At the end of experiment	24.56±1.81	28.86±2.04	16.01±1.66	26.14±1.48	0.048*

*P<0.05, **P<0.01

Table 2 Complete blood count (CBC) of mice in all groups

	Control group	*Punica granatum L.* group	Toxin group	*Punica granatum L.* + toxin group	P value
RBCs (10^6)/(uL)	7.34±0.51	7.82±0.83	7.88±0.60	7.01±0.24	0.071
Hemoglobin (g/dL)	10.28±0.56	10.90±0.74	10.18±0.55	10.01±0.42	0.077
Hematocrit (%)	41.77±0.59	41.88±0.51	38.66±0.92	38.06±0.38	0.083
MCV (fL)	50.28±0.72	50.11±0.91	48.50±0.78	48.62±0.81	0.094

*P<0.05, **P<0.01, red blood cells (RBCs), mean cell volume (MCV)

Table 3 Serum biochemical panel for all mice groups

	Control group	*Punica granatum L.* group	Toxin group	*Punica granatum L.* + toxin group	*p* value
Glucose (mmol/L)	4.30±0.32	4.6±0.21	4.80±0.57	4.91±0.42	0.821
Cholesterol (mmol/L)	2.02±0.45	1.29±0.08	2.82±0.22	1.51±0.40	0.043*
HDL-C (mmol/L)	1.54±0.62	2.69±0.73	1.02±0.44	2.28±0.44	0.041*
LDL-C (mmol/L)	2.56±0.70	1.61±0.11	2.90±0.63	2.03±0.81	0.048*
ALT (U/L)	66.71±6.28	41.89±45.09	234.60±28.61	101.27±14.71	0.003**
AST (U/L)	81.41±17.22	67.21±9.05	251.45±34.90	162.81±25.84	0.003**
ALP (U/L)	98.64±18.01	52.37±11.72	258.33±58.16	182.33±31.20	0.002**
Urea (mg/dL)	22.57±2.78	18.37±3.01	61.11±11.96	44..02±8.39	0.048*
Creatinine (mg/dL)	0.66±0.26	0.52±0.06	0.965±0.77	0.996±0.51	0.0723
CK (U/L)	191.22±15.33	176.21±9.06	423.67±52.76	356.88±33.71	0.021*
CK-MB (U/L)	39.77±2.17	30.56±3.09	165.37±20.51	92.62±10.67	0.006**
c-troponin I	6.71±0.72	5.22±0.32	9.69±2.11	8.22±5.44	0.034*

*P<0.05, **P<0.01, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine kinase (CK), creatinine kinase-MB (CK-MB).

In cardiac tissue, the GSH level was increased significantly in the *Punica granatum L* treated group.after the administration of toxins compared with the GSH level in the toxins group (p<0.05). Moreover, catalase and GSH-Px activities were significantly higher in the *Punica granatum L.* group after toxins injection than those in the toxins group (p<0.01 and p<0.05, respectively) (Table 4).

Table 4 Oxidants and antioxidants levels in cardiac tissue for all mine groups

	Control group	Punica granatum L. group	Toxin group	Punica granatum L. + toxin group	*p* value
LPO (nmol/g)	0.870±0.31	0.839±0.33	0.892±0.37	0.832±0.36	0.103
CC (nmo/gl)	0.306±0.065	0.329±0.034	0.422±0.049	0.399±0.031	0.069
GSH (nmol/g)	1.81±0.42	6.05±0.79	0.68±0.24	9.88±0.82	0.021*
Catalase (kU/g protein)	1209.62±66.41	1952.23±69.71	237.62±55.49	393.44±33.95	0.007**
GSH-Px (U/g protein)	1.16±0.45	1.44±0.61	0.79±0.35	1.00±0.41	0.048*
SOD (ug/g protein)	17.71±3.02	19.56±2.47	16.51±3.92	16.44±3.69	0.061

*P<0.05, **P<0.01, lipid peroxidation (LPO), protein carbonyl content (CC), reduced glutathione (GSH), catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD)

The histopathological study of the cardiac tissues of the toxin-induced group showed a massive haemorrhage with necrotic cardiomyocyte with no striation (Figure 1A). The heart of the *Punica granatum L.* group following the introduction of toxins, cardiomyocytes appeared normally with normal nuclei and striation were observed; nevertheless, there is a slight haemorrhage around the cardiac fibres (Figure 1B).

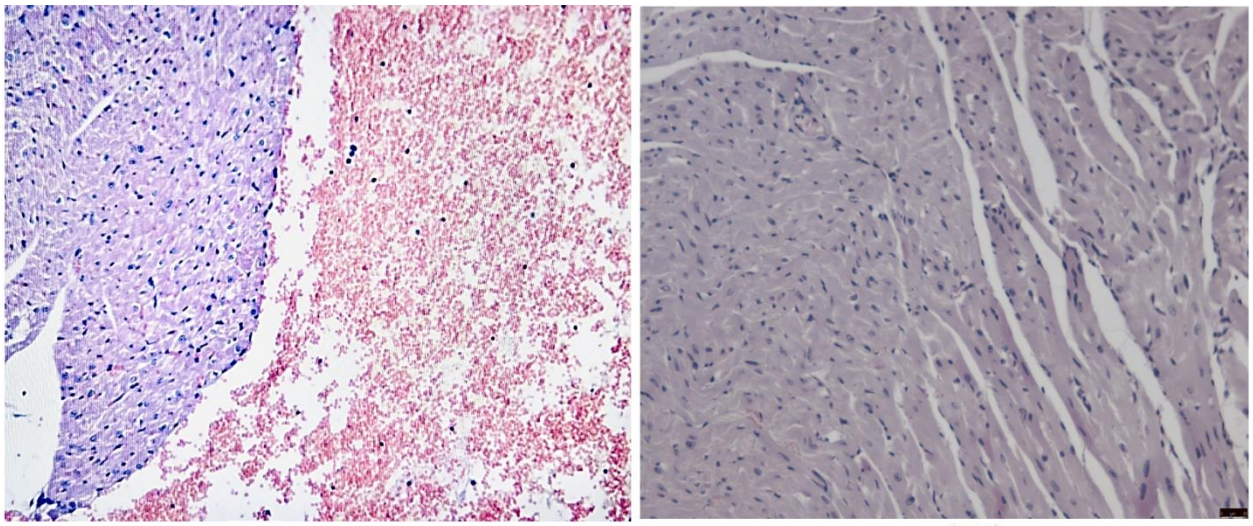

Figure 1 (A) Massive bleeding was seen in the group heart, as well as cardiomyocyte death and the lack of normal striation. (B) *Punica granatum L.* plus toxin group mice heart showed normal cardiomyocyte with normal nuclei and normal striation. There is slight hemorrhage around cardiac fibers.

In hepatic tissue, the LPO level was significantly decreased in the group treated with *Punica granatum L.* after toxins injection compared with the toxins group ($p<0.05$). Moreover, significantly high level of the GSH was determined in the group treated with *Punica granatum L.* following the administration of toxins than those in the toxins group ($p<0.01$). In addition, catalase and GSH-Px activities were significantly increased in the *Punica granatum L.* group after toxin injections compared with the toxins group ($p<0.01$ and $p<0.05$, respectively) (Table 5).

Table 5 Oxidants and antioxidants levels in hepatic tissue for all mice groups.

	Control group	*Punica granatum L.* group	Toxin group	*Punica granatum L.* + toxin group	*p* value
LPO (nmol/g)	0.533±0.39	0.411±0.32	0.979±0.37	0.853±0.40	0.035*
CC (nmol/g)	0.420±0.061	0.444±0.052	0.631±0.062	0.0.611±0.049	0.087
GSH (nmol/g)	1.32±0.48	8.53±0.83	1.12±0.24	18.28±2.06	0.005**
Catalase (kU/g protein)	531.60±33.82	1523.75±23.91	137.62±31.49	323.55±40.47	0.006**
GSH-Px (U/g protein)	1.16±0.45	1.44±0.61	0.79±0.35	1.00±0.41	0.048*
SOD (ug/g protein)	18.02±2.06	18.88±2.48	15.60±3.10	15.22±2.09	0.062

*$P<0.05$, **$P<0.01$, lipid peroxidation (LPO), protein carbonyl content (CC), reduced glutathione (GSH), catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD)

The histopathological study showed that the liver of the toxins group had inflammatory immune cell infiltration all over the hepatic central vein. In addition, hepatocyte revealed a condensed nucleus with necrotic cells and cytoplasmic vacuoles (Figure 2A). The liver of the *Punica granatum L.* and toxins groups histologically appears with a dilated central vein and normal hepatocytes. The sinusoidal space showed a slight proliferation of Kupffer cells (Figure 2B).

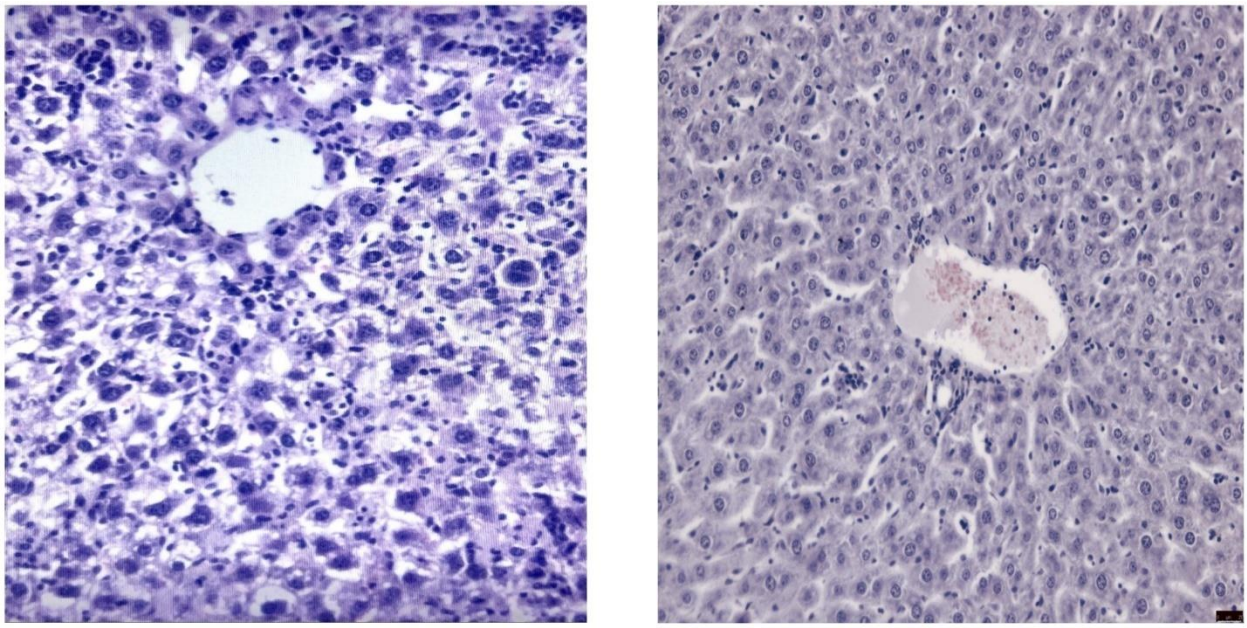

Figure 2 (2A) Immune inflammatory cell infiltration was surrounding the central vein were seen in the liver of the Toxins group. Hepatocytes have cytoplasmic vacuoles (fat droplets) and a condensed nucleus, as well as necrotic cells. (2B). *Punica granatum L.* plus toxin group mice liver showed dilated central vein with normal hepatocytes. The sinusoidal space appears normal with slight increase in Kupffer's cells.

In the kidney homogenate, there was a significant increase in the activity of catalase and GSH-Px in the *Punica granatum L.* treated group, after the administration of toxins compared with the toxins group ($p<0.01$ and $p<0.05$, respectively) (Table 6). The kidney of the toxins group appears with marked congested dilated blood vessels and hyaline casts in glomeruli and tubules (Figure 3A). After toxin injections, the kidney treated with *Punica granatum L.* showed hypercellular glomeruli with hyaline material in tubules. There is a slight haemorrhage in the interstitial tissue (Figure 3B).

Table 6 Oxidants and antioxidants levels in kidney tissue for all mice groups.

	Control group	*Punica granatum L.* group	Toxin group	*Punica granatum L.* + toxin group	*p* value
LPO (nmol/g)	0.776±0.31	0.833±0.29	0.958±0.37	0.878±0.38	0.131
CC (nmol/g)	0.338±0.061	0.371±0.055	0.427±0.079	0.444±0.031	0.058
GSH (nmol/g)	1.05±0.28	2.44±0.71	0.68±0.24	2.78±0.45	0.064
Catalase (kU/g protein)	650.35±39.72	1263.44±51.08	237.62±55.49	309.59±32.91	0.003**
GSH-Px (U/g protein)	1.16±0.45	1.44±0.61	0.79±0.35	1.00±0.41	0.048*
SOD (ug/g protein)	18.09±1.93	18.59±2.11	16.40±2.47	16.89±3.62	0.062

*$P<0.05$, **$P<0.01$, lipid peroxidation (LPO), protein carbonyl content (CC), reduced glutathione (GSH), catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD)

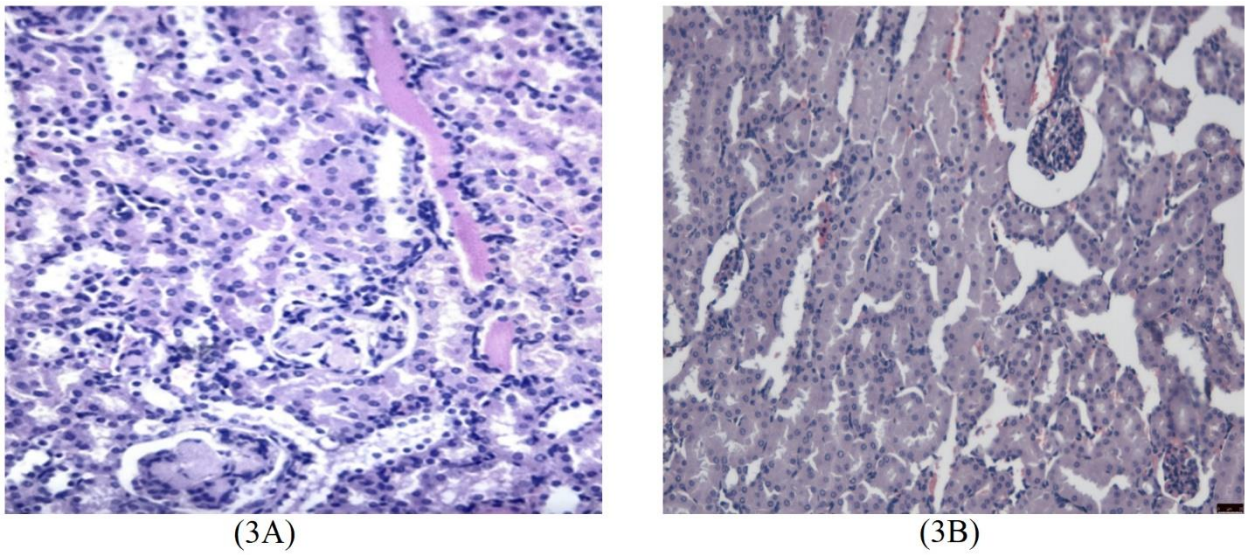

Figure 3 (3A) Toxins group kidney showed marked congested dilated blood vessel and hyaline materials in glomeruli and tubules. (3B) *Punica granatum L.* plus toxin group mice kidney showed hypercellular glomeruli with hyaline material in tubules. There is slight hemorrhage in interstitial tissue.

4. DISCUSSION

The first aim of this study was to explore the potential effect of antioxidant activities of *Punica granatum L.* peels extract on toxicity in mice induced by a mixture of acetaminophen, Nerium oleander ethanolic extract, and gentamicin. The antioxidant biomarkers selected for analysis are known to be involved in a broadspectrum of biological functions associated with oxidative stress defence at the cellular level, including inflammation, cellular stress, insulin sensitivity, adipogenesis, lipogenesis, and metabolic activities (Ceballos-Picot et al., 1996). It is widely recognised that plant-based dietary nutrients with high levels of phenols have a number of potential health benefits, and they can protect against harmful cellular events, including inflammation and the generation of ROS. *Punica granatum* contains significant quantities of phenolic compounds, mainly flavonoids, tannins, and phenolic acid, which have been demonstrated to possess a wide spectrum of biological properties as antioxidant, anti-inflammatory, anti-obesity, anti-diabetic, and hypolipidemic mediators, as well to play a role in insulin sensitisation (Poyrazoğlu et al., 2002; Mirdehghan and Rahemi, 2007; Singh et al., 2018).

Increases in body weight and fat accumulation are highly correlated with the development of hyperlipidaemia, hyperglycaemia, and insulin resistance (Eguchi et al., 2006). Upon treatment with dietary phenols, body weight reduction and an improvement in the lipid profile was noticed in obese animals under several paradigms (Fukuchi et al., 2008; Ikarashi et al., 2011). Accordingly, the primary question asked in this study was whether frequent (daily) consumption of *Punica granatum L.*would result in considerable alterations across the animal models. After the eight-week supplementation period was completed, the data obtained indicated that the daily consumption of 500 mg/kg body weight of *Punica granatum L.* extract was associated with a modest increase in the mice group that was fed with *Punica granatum*. These modest increases would be associated to the hypothetical role of *Punica granatum* L. in improving the body's capability to prevent diseases. In other words, improving the body's weight without altering the lipid profile shows a good predictor effect of the extract on one's general lifestyle. This is consistent with the literature regarding the role of *Punica granatum* in improving general health (Hossin, 2009).

Based on the influence of the intervention on the weight of the white mice with average weight in the range of 19–21 g, these findings are inconsistent with a previous study that made suggestions that are consistent with the present findings. A recent clinical trial by Aptekmannand Cesar (2013) did not demonstrate any significant difference in the body weights of healthy workers (males and females of various ages and weights) who had been consuming orange juice over a long time period (≥12 months) when compared to non-orange juice drinkers in the same sedentary environment (Aptekmann and Cesar, 2013). However, the body of evidence concerning the hypolipidaemic effect of phenolic compounds on the plasma lipid profile is more consistent compared to the effect on body weight. A great number of trials and human studies have demonstrated that long-term consumption of phenolic compounds is associated with hypercholesterolaemia, reduced total cholesterol, LDL cholesterol, and apo-B, and increased HDL-cholesterol (Kurowska et al., 2000; Roza et al., 2007). Consistent with these findings, our study demonstrated that *Punica granatum* L. peels extract supplementation was associated with a significant reduction in the plasma lipid profile, including LDL-c and

cholesterol in parallel with a dramatic increase in the HDL-c level across the group treated with *Punica granatum* L. compared to other treated groups. These findings suggested that *Punica granatum* L. induced a hypolipidemic effect. This effect might be attributed to the potential antioxidant effect of *Punica granatum* on cholesterol oxidation (Al-Muammar and Khan, 2012).

Regarding the liver function tests, our data demonstrated that *Punica granatum L*. induced a significant reduction in the liver enzymes, including ALT, AST, and ALP. Moreover, *Punica granatum L*. increased the GSH level and activity of catalase and GSH-Px in liver homogenate. The histological findings of the hepatic cells, it shows the protective effect of *Punica granatum L*. extract on liver histology against the mixture of toxins. These toxins induce hepatocyte necrosis and blood vessel congestion in the liver, which cannot be observed in the liver treated with the extract. Regarding kidney function, the *Punica granatum L*. extract reduced the urea level significantly. Moreover, the extract induced the activity of the antioxidant enzymes catalase and GSH-Px. Histologically; the toxins mixture caused marked congestion of blood vessels in the kidney. Furthermore, the toxins induced the formation of hyaline material in glomeruli and tubules. The protective effect of the *Punica granatum L*. extract did not appear in the kidney. Still, there was a hyaline material in the kidney tubules. In addition, the glomeruli showed hypercellularity with a slight haemorrhage in the interstitial tissue.

After toxins injections, the extract of *Punica granatum L*. significantly reduced the activity of cardiac enzymes and the level of cardiac troponin I. Furthermore, the extract induced the GSH level and the activity of catalase and GSH-Px. At the histological level, the extract protected the heart from the necrosis and the massive haemorrhage induced by the toxins.

5. CONCLUSION

Punica granatum L. peels induced hypolipidemic effect. This effect might be anticipated to the potential antioxidant effect against cholesterol oxidation. There is a protective effect of the extract on liver histology against mixture of toxins. Also, the extract protects the heart from necrosis and massive haemorrhage.

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author upon request.

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